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## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
United States Patent and Trademark  
Office  
Box PCT  
Washington, D.C. 20231  
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

<b>Date of mailing</b> (day/month/year) 08 June 2000 (08.06.00)	
<b>International application No.</b> PCT/US99/22725	<b>Applicant's or agent's file reference</b> DEX-0047
<b>International filing date</b> (day/month/year) 30 September 1999 (30.09.99)	<b>Priority date</b> (day/month/year) 02 October 1998 (02.10.98)
<b>Applicant</b> MACINA, Roberto, A.	

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

01 May 2000 (01.05.00)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

F. Baechler

Telephone No.: (41-22) 338.83.38

# PATENT COOPERATION TREATY

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: LICATA, JANE MASSEY  
LAW OFFICES OF JANE MASSEY LICATA  
66 E. MAIN ST  
MARLTON, NEW JERSEY 08053

**PCT**

OCT 2000

WRITTEN OPINION

(PCT Rule 66)

Docket System ☒  
Status Report ☒  
Docket Book ☒  
12/2/00 ans

Date of Mailing  
(day/month/year)

**02 OCT 2000**

Applicant's or agent's file reference  
DEX-0047

**REPLY DUE**

within **TWO** months  
from the above date of mailing

International application No.  
PCT/US99/22725

International filing date (day/month/year)  
30 SEPTEMBER 1999

Priority date (day/month/year)  
02 OCTOBER 1998

International Patent Classification (IPC) or both national classification and IPC  
IPC(7): C12Q 1/68; G01N 33/53; C07K 16/00, 16/18, 16/20 and US Cl.: 435/6, 7.1, 7.92; 530/387.1, 387.3, 387.7, 388.1, 388.8, 388.85

Applicant  
Diadexus LLC

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

**When?** See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).~~

**How?** By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

**Also** For an additional opportunity to submit amendments, see Rule 66.4.  
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.  
For an informal communication with the examiner, see Rule 66.6.

**If no reply is filed**, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 02 FEBRUARY 2001

Name and mailing address of the IPEA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

LARRY HELMS

Telephone No. (703) 308-0196

*Larry Helms*

## I. Basis of the opinion

## 1. With regard to the elements of the international application:\*

☒ the international application as originally filed☒ the description:

pages 1-22 , as originally filed  
pages NONE , filed with the demand  
pages NONE , filed with the letter of

☒ the claims:

pages 23-25 , as originally filed  
pages NONE , as amended (together with any statement) under Article 19  
pages NONE , filed with the demand  
pages NONE , filed with the letter of

☒ the drawings:

pages NONE , as originally filed  
pages NONE , filed with the demand  
pages NONE , filed with the letter of

☒ the sequence listing part of the description:

pages 1-3 , as originally filed  
pages NONE , filed with the demand  
pages NONE , filed with the letter of

## 2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

## 3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

- ☒ contained in the international application in printed form.  
☒ filed together with the international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

- ☒ the description, pages NONE  
☒ the claims, Nos. NONE  
☒ the drawings, sheets/fig NONE

5. ☐ This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 8-11

because:

☐ the said international application, or the said claim Nos. \_ relate to the following subject matter which does not require international preliminary examination (*specify*).

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. \_ are so unclear that no meaningful opinion could be formed (*specify*).

☐ the claims, or said claims Nos. \_ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 8-11.

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

**V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. statement**

Novelty (N)	Claims	<u>NONE</u>	YES
	Claims	<u>1-7</u>	NO
Inventive Step (IS)	Claims	<u>NONE</u>	YES
	Claims	<u>1-7</u>	NO
Industrial Applicability (IA)	Claims	<u>1-7</u>	YES
	Claims	<u>NONE</u>	NO

**2. citations and explanations**

Claims 1-7 lack novelty under PCT Article 33(2) as being anticipated by HUMAN GENOME SCIENCES, INC. (WO 96/39541).

The claims recite a method for diagnosing the presence of gastrointestinal cancer, metastasis, staging, and monitoring by measuring CC2 levels wherein the CC2 comprises SEQ ID NO:1 or SEQ ID NO:2.

HUMAN GENOME SCIENCES, INC. teach the amino acid sequence SEQ ID NO:2 which is identical to the CC2 amino acid sequence of SEQ ID NO:2 in the instant application. Also taught is a method to determine metastasis, comparing cancerous tissue to normal tissues. Since metastasis is a stage in cancer the limitation of staging has been met.

Claims 1-7 lack novelty under PCT Article 33(2) as being anticipated by INCYTE PHARMACEUTICALS, INC. (WO 98/16640).

The claims have been described supra.

INCYTE PHARMACEUTICALS, INC. teach that the reg protein Igama which is SEQ ID NO:1 is expressed in colon and rectal tumors but is not in normal tissue. The amino acid sequence of SEQ ID NO:1 of INCYTE PHARMACEUTICALS, INC. is identical to the amino acid sequence of CC2 of the instant application. Also taught are antibodies to the protein which can be used for diagnosis and monitoring and can be used for determining metastasis. Since metastasis is a stage in cancer, the limitation of staging has been met.

----- NEW CITATIONS -----

NONE

**Supplemental Box**  
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

**TIME LIMIT:**

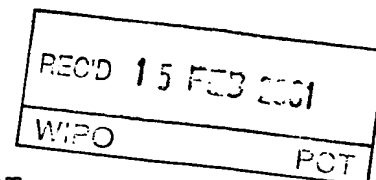
The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

TENT COOPERATION TRE

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)



Applicant's or agent's file reference DEX-0047	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US99/22725	International filing date (day/month/year) 30 SEPTEMBER 1999	Priority date (day/month/year) 02 OCTOBER 1998
International Patent Classification (IPC) or national classification and IPC IPC(7): C12Q 1/68; G01N 33/53; C07K 16/00, 16/18, 16/20 and US Cl.: 435/6, 7.1, 7.92; 530/387.1, 387.3, 387.7, 388.1, 388.8, 388.85		
Applicant DIADEXUS LLC		

- This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 5 sheets.  
☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  
These annexes consist of a total of 0 sheets.

- This report contains indications relating to the following items:
  - ☒ Basis of the report
  - ☐ Priority
  - ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability
  - ☐ Lack of unity of invention
  - ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
  - ☐ Certain documents cited
  - ☐ Certain defects in the international application
  - ☐ Certain observations on the international application

Date of submission of the demand  01 MAY 2000	Date of completion of this report  02 JANUARY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer  LARRY R. HELMS Telephone No. (703) 305-0190  TERRY J. DEY PARALEGAL SPECIALIST TECHNOLOGY CENTER 1600



## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/22725

## I. Basis of the report

## 1. With regard to the elements of the international application: \*

- ☒ the international application as originally filed
- ☒ the description:  
pages 1-22 , as originally filed  
pages NONE , filed with the demand  
pages NONE , filed with the letter of \_\_\_\_\_
- ☒ the claims:  
pages 23-25 , as originally filed  
pages NONE , as amended (together with any statement) under Article 19  
pages NONE , filed with the demand  
pages NONE , filed with the letter of \_\_\_\_\_
- ☒ the drawings:  
pages NONE , as originally filed  
pages NONE , filed with the demand  
pages NONE , filed with the letter of \_\_\_\_\_
- ☒ the sequence listing part of the description:  
pages 1-3 , as originally filed  
pages NONE , filed with the demand  
pages NONE , filed with the letter of \_\_\_\_\_

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.  
These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

## 3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in printed form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

- ☒ the description, pages NONE
- ☒ the claims, Nos. NONE
- ☒ the drawings, sheets/fig NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\*Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/22725

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 8-11

because:

☐ the said international application, or the said claim Nos. \_ relate to the following subject matter which does not require international preliminary examination (*specify*).

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. \_ are so unclear that no meaningful opinion could be formed (*specify*).

☐ the claims, or said claims Nos. \_ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 8-11.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/22725

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. statement**

Novelty (N)	Claims	<u>NONE</u>	YES
	Claims	<u>1-7</u>	NO
Inventive Step (IS)	Claims	<u>NONE</u>	YES
	Claims	<u>1-7</u>	NO
Industrial Applicability (IA)	Claims	<u>1-7</u>	YES
	Claims	<u>NONE</u>	NO

**2. citations and explanations (Rule 70.7)**

Claims 1-7 lack novelty under PCT Article 33(2) as being anticipated by HUMAN GENOME SCIENCES, INC. (WO 96/39541).

The claims recite a method for diagnosing the presence of gastrointestinal cancer, metastasis, staging, and monitoring by measuring CC2 levels wherein the CC2 comprises SEQ ID NO:1 or SEQ ID NO:2.

HUMAN GENOME SCIENCES, INC. teach the amino acid sequence SEQ ID NO:2 which is identical to the CC2 amino acid sequence of SEQ ID NO:2 in the instant application. Also taught is a method to determine metastasis, comparing cancerous tissue to normal tissues. Since metastasis is a stage in cancer the limitation of staging has been met.

Claims 1-7 lack novelty under PCT Article 33(2) as being anticipated by INCYTE PHARMACEUTICALS, INC. (WO 98/16640).

The claims have been described supra.

INCYTE PHARMACEUTICALS, INC. teach that the reg protein Igamm which is SEQ ID NO:1 is expressed in colon and rectal tumors but is not in normal tissue. The amino acid sequence of SEQ ID NO:1 of INCYTE PHARMACEUTICALS, INC. is identical to the amino acid sequence of CC2 of the instant application. Also taught are antibodies to the protein which can be used for diagnosis and monitoring and can be used for determining metastasis. Since metastasis is a stage in cancer, the limitation of staging has been met.

----- NEW CITATIONS -----

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/22725

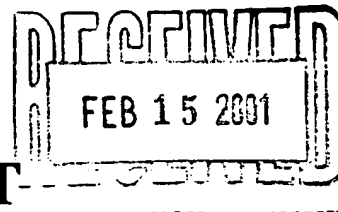
**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

# PATENT COOPERATION TREATY



From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: LICATA, JANE MASSEY  
LAW OFFICES OF JANE MASSEY LICATA  
66 E. MAIN ST  
MARLTON, NEW JERSEY 08053

Docket System ☒  
Status Report ☒  
Docket Book ☒

NP = 4.2.01

## NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing  
(day/month/year)

12 FEB 2001

Applicant's or agent's file reference

DEX-0047

### IMPORTANT NOTIFICATION

International application No.

PCT/US99/22725

International filing date (day/month/year)

30 SEPTEMBER 1999

Priority Date (day/month/year)

02 OCTOBER 1998

Applicant

DIADEXUS LLC

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

LARRY R. HELMS

Telephone No. (703) 308-0196

**TERRY J. DEY**  
**PARALEGAL SPECIALIST**  
**TECHNOLOGY CENTER 1000**

09/806311

JC08 Rec'd PCT/PTO 29 MAR 2001

**A NOVEL METHOD OF DIAGNOSING, MONITORING, STAGING,  
IMAGING AND TREATING GASTROINTESTINAL CANCERS**

**FIELD OF THE INVENTION**

This invention relates, in part, to newly developed  
5 assays for detecting, diagnosing, monitoring, staging  
prognosticating, imaging and treating cancers, particularly  
gastrointestinal cancers including cancer of the stomach,  
small intestine and colon.

**BACKGROUND OF THE INVENTION**

10 Cancer of the colon is the second most frequently  
diagnosed malignancy in the United States, as well as the  
second most common cause of cancer death. Colon cancer is  
a highly treatable and often curable disease when localized  
to the bowel. Surgery is the primary treatment and results  
15 in cure in approximately 50% of patients. However,  
recurrence and metastases following surgery is a major  
problem and often is the ultimate cause of death.

Due to its proximity, cancer of the colon often  
metastasizes to the small intestine. The prognosis of the  
20 cancer spreading to the small intestine is related to the  
degree of penetration of the tumor through the bowel wall  
and the presence or absence of nodal involvement. These  
two characteristics form the basis for all staging systems  
developed for colon cancer. Various characteristics also  
25 assist in prognosticating colon cancer and its spread to  
the small intestines. For example, bowel obstruction and  
bowel perforation are indicators of poor prognosis.  
Elevated pretreatment serum levels of carcinoembryonic  
antigen (CEA) and of carbohydrate antigen 19-9 (CA 19-9)  
30 also have a negative prognostic significance. However, age  
greater than 70 years at presentation is not a  
contraindication to standard therapies; acceptable

- 2 -

morbidity and mortality, as well as long-term survival, are achieved in this patient population.

Cancer cells can also originate in the small intestine. However, this is a much rarer type of cancer.

5        Symptoms of cancer of the small intestine typically include pain or cramps in the middle of the abdomen, weight loss without dieting, a lump in the abdomen or blood in the stool.

10        Cancer of the stomach, also referred to as gastric cancer, also frequently metastasizes to the small intestine due to its proximity. This cancer is often difficult to diagnose in early stages and can be in the stomach for a long time, growing to a large size before symptoms arise. In the early stages of cancer of the stomach, an individual  
15        may experience indigestion and stomach discomfort, a bloated feeling after eating, mild nausea, loss of appetite or heartburn. In more advanced stages of stomach cancer, there may be blood in the stool, vomiting, weight loss or more severe pain.

20        Because of the frequency of these types of cancer (approximately 160,000 new cases of colon and rectal cancer per year alone), the identification of high-risk groups, the demonstrated slow growth of primary lesions and the better survival of early-stage lesions, screening for  
25        gastrointestinal cancers should be a part of routine care for all adults starting at age 50, especially those with first-degree relatives with colorectal cancer.

30        Procedures used for detecting, diagnosing, monitoring, staging, and prognosticating cancer of the colon, small intestine or stomach are of critical importance to the outcome of the patient. Patients diagnosed with early stage cancer generally have a much greater five-year survival rate as compared to the survival rate for patients diagnosed with distant metastasized  
35        cancers. New diagnostic methods which are more sensitive

- 3 -

and specific for detecting early cancer of the stomach, small intestine and colon are clearly needed.

Patients with gastrointestinal cancers are closely monitored following initial therapy and during adjuvant  
5 therapy to determine response to therapy and to detect persistent or recurrent disease of metastasis. There is clearly a need for a cancer marker which is more sensitive and specific in detecting recurrence of these types of cancer.

10 Another important step in managing gastrointestinal cancers is to determine the stage of the patient's disease. Stage determination has potential prognostic value and provides criteria for designing optimal therapy. Generally, pathological staging of cancer is preferable  
15 over clinical staging because the former gives a more accurate prognosis. However, clinical staging would be preferred were it at least as accurate as pathological staging because it does not depend on an invasive procedure to obtain tissue for pathological evaluation. Staging of  
20 gastrointestinal cancers would be improved by identifying new markers in cells, tissues, or bodily fluids which could differentiate between different stages of invasion.

Thirteen colon specific genes and naturally occurring variants thereof, referred to as CSG1-13, are disclosed in  
25 U.S. Patent 5,733,748 and WO 96/39541 for use as diagnostic markers in colon cancer. Some of these genes and polypeptides encoded thereby are also taught to be useful in determining if the colon cancer has metastasized.

U.S. Patent 5,861,494, which issued January 19, 1999,  
30 also discloses a gene and polypeptide encoded thereby for use as a diagnostic marker for colon cancer and as an agent for determining if the colon cancer has metastasized. This gene and the polypeptide encoded thereby are similar in sequence to the cancer specific gene referred to herein as  
35 CC2.



- 4 -

It has now been found that CC2 is a useful diagnostic and metastatic marker not only for colon cancer but also for cancer of the stomach and small intestine. Thus, in the present invention, methods are provided for detecting, 5 diagnosing, monitoring, staging, prognosticating, imaging and treating gastrointestinal cancers including cancer of the stomach, small intestine and colon via the cancer specific gene referred to herein as CC2. CC2 refers, among other things, to native protein expressed by the gene 10 comprising the polynucleotide sequence of SEQ ID NO:1. The amino acid sequence of a polypeptide encoded by SEQ ID NO:1 is depicted herein as SEQ ID NO:2. In the alternative, what is meant by CC2 as used herein, means the native mRNA encoded by the gene comprising the polynucleotide sequence 15 of SEQ ID NO:1 or levels of the gene comprising the polynucleotide sequence of SEQ ID NO:1.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should 20 be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will 25 become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

#### **SUMMARY OF THE INVENTION**

Toward these ends, and others, it is an object of the 30 present invention to provide a method for diagnosing the presence of a gastrointestinal cancer by analyzing for changes in levels of CC2 in cells, tissues or bodily fluids compared with levels of CC2 in preferably the same cells, tissues, or bodily fluid type of a normal human control,

- 5 -

wherein a change in levels of CC2 in the patient versus the normal human control is associated with a gastrointestinal cancer.

Further provided is a method of diagnosing metastatic cancer in a patient having a gastrointestinal cancer which is not known to have metastasized by identifying a human patient suspected of having a gastrointestinal cancer that has metastasized; analyzing a sample of cells, tissues, or bodily fluid from such patient for CC2; comparing the CC2 levels in such cells, tissues, or bodily fluid with levels of CC2 in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein an increase in CC2 levels in the patient versus the normal human control is associated with a gastrointestinal cancer which has metastasized.

Also provided by the invention is a method of staging a gastrointestinal cancer in a human which has such cancer by identifying a human patient having such cancer; analyzing a sample of cells, tissues, or bodily fluid from such patient for CC2; comparing CC2 levels in such cells, tissues, or bodily fluid with levels of CC2 in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CC2 levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CC2 is associated with a cancer which is regressing or in remission.

Further provided is a method of monitoring a gastrointestinal cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CC2; comparing the CC2 levels in such cells, tissue, or bodily fluid with levels of CC2 in preferably the same cells,

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tissues, or bodily fluid type of a normal human control sample, wherein an increase in CC2 levels in the patient versus the normal human control is associated with a cancer which has metastasized.

5 Further provided is a method of monitoring the change in stage of a gastrointestinal cancer in a human having such cancer by looking at levels of CC2 in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample  
10 of cells, tissues, or bodily fluid from such patient for CC2; comparing the CC2 levels in such cells, tissue, or bodily fluid with levels of CC2 in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CC2 levels in the  
15 patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CC2 is associated with a cancer which is regressing or in remission.

Further provided are antibodies targeted against CC2  
20 or fragments of such antibodies which can be used to detect or image localization of CC2 in a patient for the purpose of detecting or diagnosing a disease or condition. Such antibodies can be polyclonal, monoclonal, or omnichlonal or prepared by molecular biology techniques. The term  
25 "antibody", as used herein and throughout the instant specification is also meant to include aptamers and single-stranded oligonucleotides such as those derived from an in vitro evolution protocol referred to as SELEX and well known to those skilled in the art. Antibodies can be  
30 labeled with a variety of detectable labels including, but not limited to, radioisotopes and paramagnetic metals. These antibodies or fragments thereof can also be used as therapeutic agents in the treatment of diseases characterized by expression of CC2. In therapeutic  
35 applications, the antibody can be used without or with

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derivatization to a cytotoxic agent such as a radioisotope, enzyme, toxin, drug or a prodrug.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to diagnostic assays and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging and prognosticating cancers by comparing levels of CC2 with those of CC2 in a normal human control. What is meant by levels of CC2 as used herein, means levels of the native protein expressed by the gene comprising the polynucleotide sequence of SEQ ID NO:1. The amino acid sequence of a polypeptide encoded by SEQ ID NO:1 is depicted herein as SEQ ID NO:2. In the alternative, what is meant by levels of CC2 as used herein, means levels of the native mRNA encoded by the gene comprising the polynucleotide sequence of SEQ ID NO:1 or levels of the gene comprising the polynucleotide sequence of SEQ ID NO:1. Such levels are preferably measured in at least one of cells, tissues and/or bodily fluids, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for diagnosing overexpression of CC2 protein compared to normal control bodily fluids, cells, or tissue samples may be used to diagnose the presence of cancers,

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and in particular gastrointestinal cancers. By gastrointestinal cancers it is meant to include stomach cancer, cancer of the small intestine, and colon cancer.

All the methods of the present invention may optionally include measuring levels of other cancer markers as well as CC2. Other cancer markers, in addition to CC2, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art.

#### **Diagnostic Assays**

10 The present invention provides methods for diagnosing the presence of a gastrointestinal cancer by analyzing for changes in levels of CC2 in cells, tissues or bodily fluids compared with levels of CC2 in cells, tissues or bodily fluids of preferably the same type from a normal human  
15 control, wherein a change in levels of CC2 in the patient versus the normal human control is associated with the presence of a gastrointestinal cancer.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result  
20 indicating the patient being tested has cancer is one in which cells, tissues or bodily fluid levels of the cancer marker, such as CC2, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues or bodily fluid of a  
25 normal human control.

The present invention also provides a method of diagnosing the onset of metastatic gastrointestinal cancers in a patient having a gastrointestinal cancer which has not yet metastasized. In the method of the present invention,  
30 a human cancer patient suspected of having a gastrointestinal cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a variety of means known to those of skill in the art.

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In the present invention, determining the presence of CC2 levels in cells, tissues or bodily fluid, is particularly useful for discriminating between gastrointestinal cancers which have not metastasized and gastrointestinal cancers which have metastasized. Existing techniques have difficulty discriminating between gastrointestinal cancers which have metastasized and gastrointestinal cancers which have not metastasized. However, proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker level measured in cells, tissues or bodily fluid of a human patient is CC2. The measured CC2 level in the human patient is compared with levels of CC2 in preferably the same cells, tissue or bodily fluid type of a normal human control. That is, if the cancer marker being observed is CC2 in serum, this level is preferably compared with the level of CC2 in serum of a normal human control. An increase in the CC2 in the patient versus the normal human control is associated with a gastrointestinal cancer which has metastasized.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the cancer in the patient being tested or monitored has metastasized is one in which cells, tissues or bodily fluid levels of the cancer marker, such as CC2, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues or bodily fluid of a normal patient.

Normal human control as used herein includes a human patient without cancer and/or non cancerous samples from the patient; in the methods for diagnosing or monitoring for metastasis, normal human control may preferably also include samples from a human patient that is determined by

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reliable methods to have a gastrointestinal cancer which has not metastasized.

**Staging**

The invention also provides a method of staging  
5 gastrointestinal cancers in a human patient. The method comprises identifying a human patient having such cancer and analyzing a sample of cells, tissues or bodily fluid from such human patient for CC2. In this method CC2 levels in such cells, tissues or bodily fluid are then compared  
10 with levels of CC2 in preferably the same cells, tissues or bodily fluid type of a normal human control sample, wherein an increase in CC2 levels in the human patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CC2 is  
15 associated with a cancer which is regressing or in remission.

**Monitoring**

Further provided is a method of monitoring  
gastrointestinal cancers in a human having such cancer for  
20 the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues or bodily fluid from such human patient for CC2; comparing the CC2 levels in such cells, tissues or  
25 bodily fluid with levels of CC2 in preferably the same cells, tissues or bodily fluid type of a normal human control, wherein an increase in CC2 levels in the human patient versus the normal human control is associated with a cancer which has metastasized.

30 Further provided by this invention is a method of monitoring the change in stage of gastrointestinal cancers in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample of cells, tissues or bodily  
35 fluid from such human patient for CC2; and comparing the

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CC2 levels in such cells, tissues or bodily fluid with levels of CC2 in preferably the same cells, tissues or bodily fluid type of a normal human control, wherein an increase in CC2 levels in the human patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease in the levels of CC2 is associated with a cancer which is regressing in stage or in remission.

Monitoring such patient for onset of metastasis is periodic and preferably done on a quarterly basis. However, this may be more or less frequent depending on the cancer, the particular patient, and the stage of the cancer.

#### **Assay Techniques**

Assay techniques that can be used to determine levels of gene expression (including protein levels), such as CC2 of the present invention, in a sample derived from a patient are well known to those of skill in the art. Such assay methods include, without limitation, radioimmunoassays, reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses, ELISA assays and proteomic approaches: two-dimensional gel electrophoresis (2D electrophoresis) and non-gel based approaches such as mass spectrometry or protein interaction profiling. Among these, ELISAs are frequently preferred to diagnose a gene's expressed protein in biological fluids.

An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to CC2, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to CC2. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, for example horseradish peroxidase enzyme or alkaline phosphatase.



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To carry out the ELISA, antibody specific to CC2 is incubated on a solid support, e.g. a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time CC2 binds to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to CC2 and linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to CC2. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to CC2 antibodies, produces a colored reaction product. The amount of color developed in a given time period is proportional to the amount of CC2 protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

20 A competition assay can also be employed wherein antibodies specific to CC2 are attached to a solid support and labeled CC2 and a sample derived from the host are passed over the solid support. The amount of label detected which is attached to the solid support can be correlated to a quantity of CC2 in the sample.

Nucleic acid methods can also be used to detect CC2 mRNA as a marker for gastrointestinal cancers. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASABA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse-transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an

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mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a  
5 single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR can be used to identify the presence of a specific type of cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e. gridding) can be used to detect both  
10 the expression of and quantitate the level of expression of a gene. In this approach, a cDNA encoding the CC2 gene is fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or plastic. At least a portion of the DNA encoding  
15 the CC2 gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA, isolated from the tissue of interest. Hybridization between the substrate bound DNA and the analyte can be detected and quantitated  
20 by several means including but not limited to radioactive labeling or fluorescence labeling of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of gene expression can be done by comparison of the intensity of the signal from the analyte  
25 compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and then using that material to generate a standard curve.

Of the proteomic approaches, 2D electrophoresis is a  
30 technique well known to those in the art. Isolation of individual proteins from a sample such as serum is accomplished using sequential separation of proteins by different characteristics usually on polyacrylamide gels. First, proteins are separated by size using an electric  
35 current. The current acts uniformly on all proteins, so

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smaller proteins move farther on the gel than larger proteins. The second dimension applies a current perpendicular to the first and separates proteins not on the basis of size but on the specific electric charge carried by each protein. Since no two proteins with different sequences are identical on the basis of both size and charge, the result of a 2D separation is a square gel in which each protein occupies a unique spot. Analysis of the spots with chemical or antibody probes, or subsequent protein microsequencing can reveal the relative abundance of a given protein and the identity of the proteins in the sample.

The above tests can be carried out on samples derived from a variety of cells, bodily fluids and/or tissue extracts (homogenates or solubilized tissue) obtained from a patient including those from tissue biopsies and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum or any derivative of blood.

#### ***In Vivo Antibody Use***

Antibodies which specifically bind to CC2 can also be used *in vivo* in patients suspected of suffering from gastrointestinal cancers including stomach cancer, cancer of the small intestine, and colon cancer. Specifically, antibodies which specifically bind a CC2 can be injected into a patient suspected of having a gastrointestinal cancer for diagnostic and/or therapeutic purposes. The preparation and use of antibodies for *in vivo* diagnosis is well known in the art. For example, antibody-chelators labeled with Indium-111 have been described for use in the radioimmunoscentographic imaging of carcinoembryonic antigen expressing tumors (Sumerdon et al. Nucl. Med. Biol. 1990 17:247-254). In particular, these antibody-chelators have been used in detecting tumors in patients suspected of

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having recurrent colorectal cancer (Griffin et al. J. Clin. Onc. 1991 9:631-640). Antibodies with paramagnetic ions as labels for use in magnetic resonance imaging have also been described (Lauffer, R.B. Magnetic Resonance in Medicine 5 1991 22:339-342). Antibodies directed against CC2 can be used in a similar manner. Labeled antibodies which specifically bind CC2 can be injected into patients suspected of having a gastrointestinal cancer for the purpose of diagnosing or staging of the disease status of 10 the patient. The label used will be selected in accordance with the imaging modality to be used. For example, radioactive labels such as Indium-111, Technetium-99m or Iodine-131 can be used for planar scans or single photon emission computed tomography (SPECT). Positron emitting 15 labels such as Fluorine-19 can be used in positron emission tomography. Paramagnetic ions such as Gadolinium (III) or Manganese (II) can be used in magnetic resonance imaging (MRI). Localization of the label permits determination of the spread of the cancer. The amount of label within an 20 organ or tissue also allows determination of the presence or absence of cancer in that organ or tissue.

For patients diagnosed with a gastrointestinal cancer, injection of an antibody which specifically binds CC2 can also have a therapeutic benefit. The antibody may exert 25 its therapeutic effect alone. Alternatively, the antibody may be conjugated to a cytotoxic agent such as a drug, toxin or radionuclide to enhance its therapeutic effect. Drug monoclonal antibodies have been described in the art for example by Garnett and Baldwin, Cancer Research 1986 30 46:2407-2412. The use of toxins conjugated to monoclonal antibodies for the therapy of various cancers has also been described by Pastan et al. Cell 1986 47:641-648. Yttrium-90 labeled monoclonal antibodies have been described for maximization of dose delivered to the tumor while limiting 35 toxicity to normal tissues (Goodwin and Meares Cancer

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Supplement 1997 80:2675-2680). Other cytotoxic radionuclides including, but not limited to Copper-67, Iodine-131 and Rhenium-186 can also be used for labeling of antibodies against CC2.

- 5        Antibodies which can be used in these *in vivo* methods include polyclonal, monoclonal and omniconal antibodies and antibodies prepared via molecular biology techniques. Antibody fragments and aptamers and single-stranded oligonucleotides such as those derived from an *in vitro* evolution protocol referred to as SELEX and well known to those skilled in the art can also be used.

- 10       The present invention is further described by the following examples. These examples are provided solely to illustrate the invention by reference to specific  
15       embodiments. These exemplifications, while illustrating certain aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

#### EXAMPLES

- 20       The examples were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following example can be carried out as described in  
25       standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

- 30       Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream 3' quencher dye. During PCR, the 5'-3' nuclease activity of

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Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

5       Amplification of an endogenous control was used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) was used  
10 as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the calibrator is obtained using the standard curve method  
15 or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

To evaluate the tissue distribution, and the level of CC2 in normal and tumor tissue, total RNA was extracted from normal tissues, tumor tissues, and from tumors and the  
20 corresponding matched normal tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was done using primers and Taqman probe specific to CC2. The results were analyzed using the ABI PRISM 7700 Sequence Detector and are provided in the  
25 following table. The absolute numbers are relative levels of expression of CC2 compared to the kidney (calibrator).

The absolute numbers depicted in Table 1 are relative levels of expression of CC2 in 12 normal different tissues. All the values are compared to normal kidney (calibrator).  
30 These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

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**Table 1: Relative Levels of CC2 Expression in Pooled Samples**

Tissue	NORMAL
Colon-Ascending	536
Endometrium	0
Kidney	1
Liver	10
Ovary	4
Pancreas	22
Prostate	332
Small Intestine	2539
Spleen	0.0
Stomach	2062
Testis	112
Uterus	2

The relative levels of expression in Table 1 show that the higher level of expression of CC2 mRNA is in tissues from the gastrointestinal tract, small intestine (2539), stomach (2062), and colon (536), with a lower level of expression in prostate (332), and testis (112). These results establish that CC2 mRNA expression is highly specific for gastrointestinal tissues including not only the colon but also the small intestine and stomach.

The absolute numbers in Table 1 were obtained analyzing pools of samples of a particular tissue from different individuals. They should not be compared to the absolute numbers originated from RNA obtained from tissue samples of single individuals depicted in Table 2.

The absolute numbers depicted in Table 2 are relative levels of expression of CC2 in 78 pairs of matching samples. All the values are compared to normal kidney (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

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**Table 2: Relative Levels of CC2 Expression in Pooled Samples**

	Sample ID	Tissue	Cancer Tissue	Normal Adjacent Tissue
5	StoAC93	Stomach 1	64860	279026
	Sto728	Stomach 2	0	40
	Sto758S	Stomach 3	21029	2903
	Sto915S	Stomach 4	3488	56
	StoAC99	Stomach 5	1162	330
10	Sto115S	Stomach 6	404	146
	Sto15S	Stomach 7	4636	14
	Sto17S	Stomach 8	59662	538
	Sto261S	Stomach 9	53061	8977
	Sto264S	Stomach 10	27492	84643
15	Sto27S	Stomach 11	20784	61
	Sto288S	Stomach 12	0	67
	Sto531S	Stomach 13	53192	8847
	Sto539S	Stomach 14	1492	27
	Sto542S	Stomach 15	26382	425
20	Sto610S	Stomach 16	1029	20
	Sto88S	Stomach 17	3846	12
	StoAc44	Stomach 18	1.7	78
	StoMT54	Stomach 19	971	67
	StoTA73	Stomach 20	35653	6020
25	SmI21XA	Small Intestine 1	31016	10022
	SmIH89	Small Intestine 2	645	2227
	ClnB56	Colon-Cecum 1	6816	971
	ClnAS45	Colon-Ascending 2	8757	5501
	ClnCM67	Colon-Cecum 3	2394	578
	ClnAS67	Colon-Ascending 4	1566	1198



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	ClnAS43	Colon-Ascending 5	127934	923
	ClnAS46	Colon-Ascending 6	96620	3316
	ClnAS98	Colon Ascending 7	83822	392
	ClnAS89	Colon-Ascending 8	10231	4
5	ClnTX01	Colon-Transverse 9	92	331
	ClnTX89	Colon-Transverse 10	11114	17
	ClnTX67	Colon-Transverse 11	683	189
	ClnMT38	Colon-Splenic flexure 12	0	6230
	ClnSG89	Colon-Sigmoid 13	2557	1243
10	ClnSG67	Colon-Sigmoid 14	39	132
	ClnSG33	Colon-Sigmoid 15	17080	118542
	ClnSG45	Colon-Sigmoid 16	243	80
	ClnB34	Colon-Rectosigmoid 17	130	11
	ClnCXGA	Colon-Rectum 18	790	47152
15	ClnRC67	Colon-Rectum 19	724	419
	ClnC9XR	Colon-Rectosigmoid 20	425	113
	ClnRS45	Colon-Rectosigmoid 21	42202	1117
	ClnRC01	Colon-Rectum 22	2693	99
	ClnRC89	Colon-Rectum 23	0	2402
20	Bld46XK	Bladder 1	0	0
	Bld66X	Bladder 2	15	4
	Bld32XK	Bladder 3	8.5	0.4
	Kid126XD	Kidney 1	5	5
	Kid12XD	Kidney 2	2	0
25	Kid5XD	Kidney 3	3.7	0.8
	Kid6XD	Kidney 4	4.3	0
	Kid106XD	Kidney 5	0	0.8
	Liv42X	Liver 1	2	1
	Liv15XA	Liver 2	0.2	0.7
30	Liv94XA	Liver 3	0	1.4

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	LngAC69	Lung 1	2	0
	LngBR94	Lung 2	3	0
	Lng47XQ	Lung 3	0	0
	Mam59X	Mammary Gland 1	0	0
5	MamB011X	Mammary Gland 2	0	0
	MamA06X	Mammary Gland 3	15	20
	Ovr103X	Ovary 1	4	0
	Ovr130X	Ovary 2	3	3
	Pan71XL	Pancreas 1	69458	15147
10	Pan82XP	Pancreas 2	0	0
	Pan77X	Pancreas 3	0	0
	Pan92X	Pancreas 4	4696	52
	PanC044	Pancreas 5	34	0
	Pro12B	Prostate 1	21	2
15	Pro23B	Prostate 2	23	6
	Pro13XB	Prostate 3	6	23
	Pro34B	Prostate 4	152	75
	Pro20XB	Prostate 5	112	13
	Pro65XB	Prostate 6	60	683
20	Tst39X	Testis 1	2361	17
	Endo10479	Endometrium 1	32	0
	Utr85XU	Uterus 1	0	0

0= Negative

In the analysis of matching samples, the higher levels of expression for CC2 are in stomach, small intestine, and colon. This pattern shows a high degree of specificity for gastrointestinal tissues including, not only the colon, but also the stomach and small intestine. These results confirm the tissue specificity results obtained with the panel of normal pooled samples (shown in Table 1).

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The level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were also compared. This comparison provides an indication of specificity for the cancer stage (e.g. different levels of mRNA expression in the cancer sample compared to the normal adjacent tissue). Table 2 shows overexpression of CC2 in 15 primary stomach cancer tissues compared with their respective normal adjacent (stomach samples #3, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15, 16, 17, 19, and 20). There is overexpression in the cancer tissues for 75% of the stomach matching samples tested (total of 20 stomach matching samples).

CC2 is also differentially expressed in the two tested matching samples for cancer of the small intestine. Sample #1 shows upregulation for the mRNA of CC2 in cancer, whereas sample #2, shows lower expression in cancer.

CC2 is differentially expressed in twenty-three matching samples for colon cancer. Samples #1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 13, 16, 17, 19, 20, 21 and 22 show upregulation for the mRNA of CC2 in cancer, whereas samples #9, 12, 14, 15, 18, and 23 show lower expression in the cancer sample when compared to the normal adjacent tissue.

Altogether, the high level of tissue specificity for gastrointestinal tissues, plus the mRNA differential expression in several of the primary stomach, small intestine, and colon matching samples tested indicate CC2 to be a diagnostic marker for gastrointestinal cancers including not only colon cancer, but also stomach cancer and cancer of the small intestine.

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What is claimed is:

1. A method for diagnosing the presence of a gastrointestinal cancer in a patient comprising:

- (a) measuring levels of CC2 in cells, tissues or  
5 bodily fluids in a patient; and
- (b) comparing the measured levels of CC2 with levels of CC2 in cells, tissues or bodily fluids from a normal human control, wherein a change in measured levels of CC2 in said patient versus normal human control is associated  
10 with the presence of a gastrointestinal cancer.

2. A method of diagnosing metastases of a gastrointestinal cancer in a patient comprising:

- (a) identifying a patient having a gastrointestinal cancer that is not known to have metastasized;
- 15 (b) measuring CC2 levels in a sample of cells, tissues, or bodily fluid from said patient; and
- (c) comparing the measured CC2 levels with levels of CC2 in cells, tissue, or bodily fluid of a normal human control, wherein an increase in measured CC2 levels in the  
20 patient versus the normal human control is associated with a cancer which has metastasized.

3. A method of staging a gastrointestinal cancer in a patient having a gastrointestinal cancer comprising:

- (a) identifying a patient having a gastrointestinal  
25 cancer;
- (b) measuring CC2 levels in a sample of cells, tissue, or bodily fluid from said patient; and
- (c) comparing measured CC2 levels with levels of CC2 in cells, tissues, or bodily fluid of a normal human  
30 control, wherein an increase in measured CC2 levels in said patient versus the normal human control is associated with a cancer which is progressing and a decrease in the

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measured CC2 levels is associated with a cancer which is regressing or in remission.

4. A method of monitoring a gastrointestinal cancer in a patient for the onset of metastasis comprising:

5 (a) identifying a patient having a gastrointestinal cancer that is not known to have metastasized;

(b) periodically measuring levels of CC2 in samples of cells, tissues, or bodily fluid from said patient; and

10 (c) comparing the periodically measured CC2 levels with levels of CC2 in cells, tissues, or bodily fluid of a normal human control, wherein an increase in any one of the periodically measured CC2 levels in the patient versus the normal human control is associated with a cancer which has metastasized.

15 5. A method of monitoring a change in stage of a gastrointestinal cancer in a patient comprising:

(a) identifying a patient having a gastrointestinal cancer;

20 (b) periodically measuring levels of CC2 in cells, tissues, or bodily fluid from said patient; and

(c) comparing the periodically measured CC2 levels with levels of CC2 in cells, tissues, or bodily fluid of a normal human control, wherein an increase in any one of the periodically measured CC2 levels in the patient versus the  
25 normal human control is associated with a cancer which is progressing in stage and a decrease is associated with a cancer which is regressing in stage or in remission.

6. The method of claim 1, 2, 3, 4 or 5 wherein the CC2 comprises SEQ ID NO:1 or SEQ ID NO:2.

30 7. An antibody which specifically binds CC2.

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8. A method of imaging a gastrointestinal cancer in a patient comprising administering to the patient an antibody of claim 7.

9. The method of claim 8 wherein said antibody is  
5 labeled with paramagnetic ions or a radioisotope.

10. A method of treating a gastrointestinal cancer in a patient comprising administering to the patient an antibody of claim 7.

11. The method of claim 10 wherein the antibody is  
10 conjugated to a cytotoxic agent.

## SEQUENCE LISTING

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<110> Macina, Roberto A.  
DIADEXUS LLC

<120> A Novel Method of Diagnosing, Monitoring, Staging,  
Imaging and Treating Gastrointestinal Cancers

<130> DEX-0047

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<150> 60/102,879

<151> 1998-10-02

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&lt;210&gt; 2

&lt;211&gt; 153

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 2

Met Arg Leu Leu Leu Leu Ser Cys Leu Ala Lys Thr Gly Val Leu  
 1 5 10 15

Gly Asp Ile Ile Met Arg Pro Ser Cys Ala Pro Gly Trp Phe Tyr His  
 20 25 30

Lys Ser Asn Cys Tyr Gly Tyr Phe Arg Lys Leu Arg Asn Trp Ser Asp  
 35 40 45

Ala Glu Leu Glu Cys Gln Ser Tyr Gly Asn Gly Ala His Leu Ala Ser  
 50 55 60

Ile Leu Ser Leu Lys Glu Ala Ser Thr Ile Ala Glu Tyr Ile Ser Gly  
 65 70 75 80

Tyr Gln Arg Ser Gln Pro Ile Trp Ile Gly Leu His Asp Pro Gln Lys  
 85 90 95

Arg Gln Gln Trp Gln Trp Ile Asp Gly Ala Met Tyr Leu Tyr Arg Ser  
 100 105 110

Trp Ser Gly Lys Ser Met Gly Gly Asn Lys His Cys Ala Glu Met Ser  
 115 120 125

Ser Asn Asn Asn Phe Leu Thr Trp Ser Ser Asn Glu Cys Asn Lys Arg  
 130 135 140



Gln His Phe Leu Cys Lys Tyr Arg Pro  
145 150

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/22725

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C12Q 1/68; G01N 33/53; C07K 16/00, 16/18, 16/30

US CL : 435/6, 7.1, 7.92; 530/387.1, 387.3, 387.7, 388.1, 388.8, 388.85

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 7.92; 530/387.1, 387.3, 387.7, 388.1, 388.8, 388.85

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E, X	US 5,861,494 A (SOPPET ET AL) 19 January 1999(19/01/99), see abstract; Figure 1.	1-7
X	WO 96/39541 A1 (HUMAN GENOME SCIENCES, INC) 12 December 1996(12/12/96), see abstract; pages 3-4; SEQ ID NO:2; page 11; pages 14-17.	1-7
X	WO 98/16640 A1 (INCYTE PHARMACEUTICALS, INC) 23 April 1998(23/04/98), see page 3, lines 7-8; pages 4-5; SEQ ID NO:1; pages 27-33.	1-7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

18 JANUARY 2000

Date of mailing of the international search report

10 FEB 2000

Name and mailing address of the ISA/US  
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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/22725

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:  
1-7
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/22725

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

CAPLUS, MEDLINE, USPATFUL, GENESEQ, PIR60, EMBL

search terms: CC2, gastrointestinal tumor, stomach tumor, digestive system, CC 2, cancer, tumor, tumour, SEQ ID NO:1, SEQ ID NO:2, antibody

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1, 2, 4, 6, and 7, drawn to a method of diagnosing metastasis of a gastrointestinal cancer of a patient and an antibody which binds CC2.

Group II, claim(s) 3 and 5, drawn to a method of staging a gastrointestinal cancer in a patient.

Group III, claim(s) 8 and 9, drawn to a method of imaging a gastrointestinal cancer in a patient.

Group IV, claim(s) 10 and 11, drawn to a method of treating gastrointestinal cancer in a patient.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Group I recites the special technical feature of an antibody and a method for diagnosing the presence of a gastrointestinal cancer. Group II recites the special technical feature of a method of staging a gastrointestinal cancer. Group III recites the special technical feature of a method of imaging in vivo a gastrointestinal cancer. And Group IV recites the special technical feature of an in vivo method of treatment of a gastrointestinal cancer. Thus, Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1.